Cerebral complications in infective endocarditis

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Till Peter, Julius, Elvira och Nicholas

"Du blir aldrig färdig, och det är som det skall"
Tomas Tranströmer

Abstract

Infective endocarditis (IE) is a life-threatening disease. Cerebral embolization complicates the course in 10-40% of IE episodes. Aims of study were to investigate the frequency of cerebrovascular complications (CVC) in left-sided IE and the influence of protective and risk factors with focus on antiplatelet and anticoagulant therapy.

CVC rate was examined by repeated magnetic resonance imaging of the brain and by assaying levels of brain damage markers in cerebrospinal fluid in 60 IE patients in paper I. The overall CVC frequency was 65%, with 35% of the patients experiencing neurological symptoms and 30% characterized as having clinically silent CVC. The risk of neurological deterioration during cardiac surgery after established cerebral embolism was low.

In paper II the relationship between symptomatic CVC and established use of antiplatelet therapy was evaluated in 684 definite left-sided IE episodes. Antiplatelet agents were used by 23% of the patients. These patients were older and more often had a history of congestive heart failure. In 25% of all episodes a CVC was seen. There was no statistically significant difference in CVC rate between patients with and without previously established antiplatelet therapy (24% vs. 25%, n.s.). Twelve-month mortality was significantly higher for patients on previously established antiplatelet therapy in the univariable analysis (34% vs. 24%, OR 1.6, 95% CI 1.1-2.4), but after adjustment for covariables the use of antiplatelet therapy was no longer a risk factor.

The association between ongoing warfarin therapy and CVC incidence in native valve endocarditis (NVE) was analyzed in paper III. Out of 587 NVE episodes 8% were seen in patients using warfarin on admission. Patients on warfarin suffered from CVC significantly less frequently than patients not on warfarin (6% vs. 26%, 0.2 95% CI 0.06-0.6). In a multivariable model *S. aureus* etiology (adjusted OR [aOR] 6.3, 95% CI 3.8-10.4) and vegetation length (aOR 1.04, 95% CI 1.01-1.07) were associated with higher CVC frequency. Warfarin use (aOR 0.26, 95% CI 0.07-0.94), history of congestive heart failure (aOR 0.22, 95% CI 0.1-0.52) and previous IE episode (aOR 0.1, 95% CI 0.01-0.79) conferred a lower risk of CVC. Cerebral hemorrhagic complications were few.

Keywords: Infective endocarditis (IE), cerebral embolism, cerebrovascular complications, antiplatelet therapy, anticoagulation, warfarin, vegetation, *Staphylococcus aureus*, mortality

List of papers

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I Snygg-Martin, U., Gustafsson, L., Rosengren, L., Alsiö, Å., Ackerholm, P., Andersson, R., Olaison, L. (2008) Cerebrovascular complications in patients with left-sided infective endocarditis are common: a prospective study using magnetic resonance imaging and neurochemical brain damage markers. *Clin Infect Dis,* **47**(1): 23-30.

II Snygg-Martin, U., Rasmussen, R.V., Hassager, C., Bruun, N.E., Andersson, R., Olaison, L. (2009) The relationship between cerebrovascular complications and established use of antiplatelet therapy: A cohort study of the effects in left-sided infective endocarditis. Submitted 2009.

III Snygg-Martin, U., Rasmussen, R.V., Hassager, C., Bruun, N.E., Andersson, R., Olaison, L. (2009) The influence of warfarin treatment on cerebrovascular complications in left-sided native valve infective endocarditis. Submitted 2009.

Contents

Abbreviations

aOR	Adjusted odds ratio
ASA	Acetylsalicylic acid
CHF	Congestive heart failure
CI	Confidence interval
CNS	Central nervous system
CoNS	Coagulase-negative staphylococci
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computed tomography
CVC	Cerebrovascular complication(s)
GFAP	Glial fibrillary acidic protein
HACEK	*Haemophilus* spp, *Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella* spp
IE	Infective endocarditis
INR	International normalized ratio
IQR	Interquartile range
IVDU	Intravenous drug use
MRI	Magnetic resonance imaging
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NFL	Neurofilament protein - light chain
NVE	Native valve endocarditis
OR	Odds ratio
PVE	Prosthetic valve endocarditis
ROC	Reciever operating characteristic
SD	Standard deviation
TEE	Transesophageal echocardiography
TIA	Transient ischemic attack(s)
TTE	Transthoracic echocardiography

1 Introduction

1.1 Infective endocarditis

Infective endocarditis (IE) is a serious disease, universally fatal if unrevealed and untreated. IE can be defined as an endovascular microbial infection of cardiac structures and intracardiac foreign bodies. The commonly accepted pathogenetic theory is that IE results from a complex interaction between initially hemodynamically derived mechanical damage to the endocardial lining, preferably on the valvular cusps, and transient bacteremia with microorganism adhesion and escape from host defense mechanisms (1, 2). Direct contact between blood and subendothelial components results in platelet and fibrin deposition and the formation of a coagulum. Circulating microorganisms adhere to the coagulum and promote further enlargement of this infected structure, referred to as the vegetation (3, 4). These irregular excrescences on the cardiac valves or endocardium, composed of platelets, fibrin, microorganisms, and inflammatory cells, are characteristic but not pathognomonic of IE. The detection of vegetations by echocardiography constitutes one of the two major clinical criteria for IE, according to the Duke and modified Duke criteria (5, 6) (table 1)**.** Continuous release of bacteria from the vegetations gives rise to the persistent bacteremia, the other cornerstone of IE.

Microorganisms adherent to cardiac valves have been shown to avoid host defense mechanisms inside the vegetation through impaired infiltration of phagocytes into the vegetation (i.e. localized agranulocytosis) (7, 8), reduced phagocytic ability (4) and protection from the humoral immune response (9). Local extension of the infection, tissue damage and embolic spread of infected material from valvular vegetations to distant organs give rise to the main complications of IE, congestive heart failure and embolic events. Causative microorganisms in IE are predominantly gram-positive bacteria, although a wide variety of pathogens can be found including gram-negative bacteria, fungi and rickettsiae. Viridans group streptococci and *S. aureus* are each found in approximately one third of IE episodes, enterococci in 10-15%, and in 5-15% of episodes blood cultures are negative.

Table 1. Definition of infective endocarditis according to the modified Duke Criteria

Definite infective endocarditis

Pathologic criteria

- Microorganisms demonstrated by results of cultures or histological examination of a vegetation, a vegetation that has embolized, or an intracardiac abscess specimen.
- Pathologic lesions; vegetation, or intracardiac abscess confirmed by histologic examination showing active endocarditis.

Clinical criteria

- 2 major criteria
- 1 major criterion and 3 minor criteria
- 5 minor criteria

Possible infective endocarditis

- 1 major criterion and 1 minor criterion
- 3 minor criteria

Rejected

- Firm alternate diagnosis explaining evidence of infective endocarditis.
- Resolution of infective endocarditis syndrome with antibiotic therapy for ≤ 4 d.
- No pathologic evidence of infective endocarditis at surgery or autopsy, with antibiotic therapy for ≤ 4 d.
- Does not meet criteria for possible infective endocarditis.

Major criteria

Blood culture findings positive for infective endocarditis

- Typical microorganisms consistent with IE from 2 separate blood cultures: Viridans streptococci, *Streptococcus bovis*, HACEK group, or *Staphylococcus aureus.* Community-acquired enterococci, in the absence of a primary focus.
- Microorganisms consistent with IE from persistently positive blood cultures (at least 2 positive cultures of blood samples drawn >12 h apart; or 3 or most of > 4 separate cultures of blood (with first and last sample drawn > 1 h apart).
- Single positive blood culture for *Coxiella burnetii* or antiphase I IgG antibody titer > 1:800).

Evidence of endocardial involvement (echocardiographic findings positive for IE)

- Oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation.
- Abscess.
- New partial dehiscence of prosthetic valve.
- New valvular regurgitation.

Minor criteria

- Predisposition: predisposing heart condition, or intravenous drug use.
- Fever, temperature ≥ 38°C.
- Vascular phenomena: major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages, Janeway´s lesions.
- Immunologic phenomena: glomerulonephritis, Osler´s nodes, Roth´s spots, rheumatoid factor.
- Microbiological evidence: positive blood culture but does not meet a major criterion*.

* Excludes positive cultures for coagulase-negative staphylococci and organisms that do not cause IE

1.2 Historical aspects

One of the first descriptions of IE dates back to 1646 and was by Rivière, who compared the autopsy findings of cardiac vegetations with "a cluster of hazel nuts". The term "vegetation" was first used by Corvisart in 1806, and in 1852 Kirkes attributed systemic emboli to vegetations on heart valves. Virchow defined this phenomenon as embolism, and in 1881 Netter described the novel practice of culturing blood from patients with endocarditis. In 1885, Osler described the triad of preexisting "chronic valvulitis", irregular fever, and embolism in his famous "Gulstonian Lectures on Malignant Endocarditis" (10), which later established his reputation as the father of the clinical entity endocarditis (11). In the 1930s the first attempts to treat IE with the antimicrobial agent sulphonamide were performed, but not until penicillin became available for clinical use in 1942 could successful treatment be achieved. It was first published by Loewe et al. in 1944 (12) and, interestingly, this and his next report (13) were both on the combination therapy of penicillin and heparin. In subsequent studies of IE heparin was associated with a high risk of cerebral hemorrhage (14-16) and anticoagulation proved unnecessary when adequate penicillin doses were used (17). The clinical diagnosis of IE was thus possible for more than 50 years before effective treatment could be given. The medical student Alfred S. Reinhardt described the patient perspective on this diagnosis in 1931 in the patient record he kept on himself when he sufferd from IE during the last 6 months of his life (figure 1).

Reinhardt's illness

- Aortic insufficiency after rheumatic fever by the age of 13. Blood pressure 160/00. Cor bovinum.
- April: Acute tonsillitis develops.
- May: Salvos of palpitations occur on exertion; petechial shower is observed on left arm.
- July: Pain develops in right knee; Reinhart is admitted to Peter Bent Brigham Hospital July 23.
- August: Blood culture is positive for Streptococcus viridans Aug. 2. Splenic infarction occurs Aug. 24.
- September: Reinhart is transferred to Boston City Hospital Sept. 11. Enlarging spleen, anemia, painful cutaneous nodules, showers of petechiae, painful muscles and joints are noted. Another splenic infarction occurs Sept 25.
- October: Attacks of transient aphasia begin Oct. 18. Right hemiplegia develops Oct. 21. On Oct. 26 pulmonary edema develops and Reinhart dies.
-

Freely based on K. M. Flegel, Dept of Medicine, McGill University, Montreal,Canada

Figure 1. Summary of the patient record of Alfred S. Reinhardt during 1931 (18)

1.3 Epidemiology

The annual incidence of IE in Sweden is estimated to be 5.9 cases per 100,000 inhabitants (19), as compared with estimated incidences of 1.9 to 9.6 cases per 100,000 individuals found in other studies (20-27). The incidence of IE is thought to have remained stable over the last three decades (20, 28) but international data indicate that the epidemiology of IE is changing. In recent studies, IE patients are found to be older, have more prosthetic valve (PVE) or device associated IE episodes, and more often undergo cardiac surgery during the period of antibiotic treatment (19, 20, 29-33). A shift toward a higher frequency of *S. aureus* IE has both been reported (34, 35) and also questioned (28). These diverging data mirror the influence of referral bias as well as differences in living conditions, genetic factors and bacterial resistance among different IE populations studied (34, 36, 37).

In-hospital mortality in patients with IE reported to the National Swedish Endocarditis Registry 1995-2007 was 12% (pers comm L. Olaison). Similar in-hospital mortality rates of 10–18% have been found in other studies (21, 26, 38-42). Mortality rate was reduced after the introduction of thoracic surgery in the 1960s and 70s while reports on mortality in IE during the last two to three decades yield conflicting results (28, 29, 39, 43). This might reflect the contemporary epidemiological changes towards older ages, more comorbid conditions, device-related episodes and more virulent microorganisms (25).

1.4 Gender aspects

In most published studies IE occurs more frequently in men than in women (22, 29, 35, 44), while female dominance was found in a prospective cohort study in Gothenburg 1984-88 (19). Results from some earlier studies of IE also show more even sex distribution (5, 45-47) while in Osler's original report there was male predominance (10). Several studies indicate that women diagnosed with IE are older than men (19, 35) while data on age is not reported in most publications. In most studies cerebral complications are reported in similar frequencies in both men and women with IE (47-52), while frequencies are not presented as separate sex data in other studies (53, 54). In one study, Roder et al. found a trend towards more neurological manifestations in women with *S. aureus* IE than in men with *S. aureus* IE (55), and *S. aureus* etiology has been found more often in women than in men with IE (34, 46). Lower surgery rates and/or higher in-hospital mortality have been reported among women as compared with men (50, 56-58) but interpretation was confounded by differences in age and comorbid conditions in some studies.

It is not known whether the observed demographic and clinical differences between women and men with IE reflect varying rates of comorbid conditions or inherent physiological differences, but may partly be explained by diagnostic, referral, or treatment bias (19). Other potential explanations have been extrapolated from animal models, suggesting that estrogen is protective against endothelial damage (59). In addition, human studies have shown that women are less likely than men to develop sepsis after traumatic hemorrhagic shock (60). Women also tend to develop heart disease later in life than men (61, 62).

1.5 Embolic events

Embolization from vegetations is a characteristic feature of IE, and complicates the course in 20-60% of IE episodes (58, 63-66), with the highest numbers seen in studies where asymptomatic embolism has also been investigated. Embolic events can cause distinct symptoms, but silent embolism has also been well documented (63, 66). The incidence of embolism has not been possible to define with certainty. The most frequent localization of embolism in left-sided IE is the cerebral vascular bed, which is involved in up to 70% (67) of embolic events. Right-sided IE typically embolizes to the lungs (68, 69). A considerable number of silent embolic events have been reported to involve the spleen and the kidneys (63). Embolic events, especially cerebral emboli, have generally been considered to have deleterious implications on the prognosis in IE (70, 71).

1.6 Neurological complications

The majority of neurological complications are established before IE is diagnosed (47, 49, 53, 63, 72). The rate of new embolic events (cerebral and peripheral) has been shown to decrease rapidly after the initiation of effective antibiotic therapy (27, 48, 73, 74). Factors predictive of embolization are not fully understood but higher incidence of embolic events has been correlated in different studies including adult IE patients, to presence of vegetations on echocardiography as well as to vegetation size and mobility characteristics, *S. aureus* etiology, mitral valve involvement, younger age and higher CRP levels (49, 54, 63-65, 75-79). Most studies also reveal higher case fatality rates in IE episodes complicated by neurologic events (10, 47, 49, 55, 72, 80-83).

Central nervous system (CNS) symptoms in IE are variable, and attempts to divide these manifestations into different pathogenetic categories have been made: embolization causing ischemic and/or hemorrhagic infarction, pri-

mary intracerebral bleeding, subarachnoid bleeding, mycotic aneurysm, infection of the brain or meninges and toxic or immune-mediated injury, encephalopathy and psychiatric manifestations (47, 84-87). It is well documented that IE patients with cerebral involvement often exhibit more than one type of lesion and/or neurological sign (47, 88-91) although they may also be asymptomatic (56, 66, 72). In a carefully described material of 17 patients dying from IE in the 1930s, the fundamental pathological change in the brain was diffuse embolic meningoencephalitis, from which various clinical manifestations arose (92). Pruitt et al. postulated that cerebral infarction, micro- and macroabscess formation, septic vasculitis and mycotic aneurysms represent a continuum, and that the advent of computed tomography (CT) and magnetic resonance imaging (MRI) has made the clinical distinction between syndromes less clear (93). Encephalopathy with impaired consciousness or delirium and meningism have also been argued to be of septic embolic origin (77, 94). Lerner concluded that infected emboli account for all neurological complications in IE (mycotic aneurysm, intracerebral bleeding, meningitis/meningoencephalitis, and brain abscess) additional to causing pure ischemic lesions (95). Roder et al. arrived at the opinion that the attempt to prove the precise nature of a neurological complication is inherently connected with difficulties (55) .

Ischemic infarction is the most common neurological complication in IE occurring in 9–28% of all IE episodes (47-49, 54, 72, 78). Large emboli preferably lodge in the middle cerebral artery territory, resulting in hemiparetic symptoms of varying degree. The clinical syndromes seen with microembolic punctuate cerebral infarctions are variable, often referred to as an altered level of consciousness or embolic encephalopathy. About half of IE episodes with cerebral emboli are described as having concomitant peripheral emboli (47). Intracranial hemorrhage occurs in 2-7% of patients with IE (47, 49, 54, 72, 82, 91, 96) and three different mechanisms are thought to prevail, namely rupture of mycotic (infectious) aneurysm, pyogenic arteritis and hemorrhagic transformation of initially bland infarction. Anticoagulant therapy has been associated with a higher risk of hemorrhagic complications in IE (47, 88, 96, 97).

The incidence of meningitis in IE varies from 3-16% in different studies and is, when it occurs, an early feature of the complex clinical manifestations of IE. Meningitis is most frequently seen with *S. aureus* as the causative pathogen, but also in IE caused by other pathogens. The detected rate of meningitis in different studies depends to a large extent on the frequency of lumbar punctures performed in the specific study setting. More than one third of patients with meningitis experience additional neurological complications (98). The availability of better and non-invasive brain imaging methods have reduced the proportion of IE patients examined by lumbar puncture and

cerebrospinal fluid (CSF) analysis, since isolated meningism is seldom the only initial neurological symptom. This is illustrated by two studies by Pruitt et al. made at different time periods, the first with IE patients from 1964 to 1973 when 85% of the patients with neurological symptoms underwent lumbar puncture, the second with patients from 1988 to 1992, when the corresponding figure was 43%. In the first study, the incidence of CSF anomalies indicative of meningitis was 16% of all IE cases, in the second it was 4% (47, 93). CSF culture is positive only in a minority of patients with IE associated meningitis (47, 49, 55, 82). Aseptic meningitis, often with moderately increased numbers of white blood cells in the CSF is thought to prevail, although this concept has been questioned (92, 98).

It is widely accepted that mortality in patients with IE is reduced in a considerable subset of patients by early surgical intervention (80, 99-102). The safety of cardiac surgery in IE patients suffering from cerebral complications is an important question since cardiopulmonary bypass is suspected to aggravate preexisting neurological deficits and to carry an increased risk of secondary cerebral hemorrhage due to heparinization. In the absence of a hemorrhagic cerebral manifestation, valve replacement has been considered resonably safe when performed at least 72 h after the cerebral event, while patients with a recent hemorrhagic cerebral complication are anticipated to have an unacceptably high risk of intracranial bleeding during early cardiac surgery (103). In a study by Ruttmann et al. (52) the suspected risk of secondary hemorrhage in IE patients undergoing cardiac surgery despite preoperatively established cerebral embolism was very low, and the authors recommended early surgical procedure for IE when indicated also after stroke.

1.7 Antiplatelet therapy

The pharmacological effects of salicylic acid have been known since ancient times. Hippocrates, the father of modern medicine, made the first recorded descriptions of the therapeutic benefits of extract of willow bark in the fifth century B.C. Salicylate-rich willow bark extract was used for its analgesic and anti-rheumatic effects. By the end of the nineteenth century German chemists had managed to synthesize acetylsalicylic acid (ASA) by acetylation of salicylic acid, and this drug, subsequently named aspirin, soon became the most sold medication worldwide. In 1908 ASA was introduced in the Swedish Pharmacopeia and became a formal part of Swedish pharmacological history.

The analgesic, antipyretic and anti-inflammatory effects of ASA were known long before its mechanisms of action through inhibition of cyclooxygenase in the prostaglandin synthesis was elucidated by Vane and coworkers

in the late 1960s (104). The antiplatelet era of ASA began with the observation that patients with regular ASA use had fewer heart attacks than expected, and later the use of ASA to reduce the rate of stroke and myocardial infarctions was approved. This effect is mediated by irreversible inhibition of cyclooxygenase and termination of production of tromboxan in platelets, and requires lower doses than used to produce the analgesic and anti-inflammatory effects. ASA reduces the relative risk of stroke, myocardial infarction and vascular death by 25% in high risk patients and has widespread use as primary and secondary prophylaxis (105).

The potential role of antiplatelet agents as adjunctive therapy in IE has provoked considerable interest, and a complex pattern of mechanisms involving platelets in the evolution of IE has been outlined (106-108). Advantageous effects of these agents have been shown under experimental conditions in several studies mainly concerning *S. aureus* IE (109-113). Other studies have failed to demonstrate such positive effects (114, 115). The results of prospective human treatment studies (116, 117) and recently published cohort studies (51, 53, 118, 119) are contradictory. Occasional reports of antiplatelet drug use in neonatal IE (120) and bovine IE (121) have opened up for further hypothesizing, but international treatment guidelines on IE do not generally include comments concerning the use of antiplatelet agents.

Newer antiplatelet drugs have been developed, of which clopidogrel has been shown to be significantly more effective than ASA in reducing the risk of stroke, myocardial infarction, or vascular death in patients with atherosclerotic vascular disease (122). Clopidogrel blocks activation of platelets by another mechanism and has fewer upper gastrointestinal side effects than ASA. Bleeding, a common complication with antiplatelet drugs irrespective of mechanism of action, has also proven to be less frequent with clopidogrel.

1.8 Anticoagulant therapy

Heparin

In 1916 McLean first observed that an extract of dog liver contained a substance that retarded the coagulation of blood in vitro. This substance was named “heparin” by Howell in 1918 and was tried in humans in 1935 (123). Its subsequent clinical use in the prevention and treatment of venous thromboembolism was introduced early in Sweden (124). In 1941, McLean evaluated the use of heparin in IE patients (125) and found that heparin treatment did not lead to improvments and resulted in a high proportion of fatal cerebral hemorrhages. Contrary to this were the results of combination therapy with heparin and penicillin found to be succesful by Loewe et al. (12). In still other studies the addition of anticoagulant therapy proved unnecessary

when adequate penicillin doses were used (45). Most authors (85, 126) have since discouraged the routine use of anticoagulant therapy in patients with IE.

Heparin is a naturally occurring glycosaminoglycan, the main function of which is to inhibit the coagulation of blood by activating antitrombin III. Subsequent inactivation of the coagulation cascade is seen mainly through inhibition of thrombin (factor II) and factor X. Low molecular weight heparin is composed of depolymerized heparin salts with lower molecular weight, longer biological half-life and more predictable effects on the coagulation system. Both unfractionated and low molecular weight heparins are contraindicated during septic endocarditis according to Swedish pharmaceutical recommendations (www.fass.se), owing to the risk of cerebral bleeding during IE.

Coumarin

In the early 1920s a previously unknown disease in cattle was recognized in the United States and Canada. The animals died of uncontrollable bleeding from minor injuries or internal hemorrhage. The cause of this condition proved to be ingestion of spoiled sweet clover *(Melilotus officinalis*) that functioned as a potent anticoagulant. The affected cattle had prolonged clotting time, owing to profound prothrombin deficiency. Coumarin was, at this time, a known component of sweet clover, causing the combination of a sweet smell and a bitter taste, and it was commercially used to scent cheap tobacco, artificial vanilla and perfumes. Not until 1939, when Karl Link and associates isolated the hemorrhagic agent from sweet clover, the association between coumarin and sweet clover disease was revealed (127). Coumarin itself , like other natural coumarins, is atoxic, but in moldy hay it is oxidized to hydroxycoumarin and linked to another coumarin molecule, thus forming a dicoumarol with potent anticoagulant effects. This compound was subsequently synthesized and the clinical use of dicoumarol began in 1941. As early as 1948 a randomized study sponsored by the American Heart Association concluded the beneficial effects of dicoumarol in myocardial infarction (128). Dicoumarol was, however, a weak and unpredictable drug and another more potent coumarin, given the name warfarin, was patented 10 years later. The initial use of warfarin was as a rat poison but the substance soon proved more reliable than dicoumarol in the clinical setting and was commercially introduced in 1954.

Warfarin is a synthetic anticoagulant substance and works through competitive inhibition of vitamin K reductase. This enzyme recycles oxidated vitamin K after vitamin K has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin (factor II), factors VII, IX and X, as well as other proteins involved in the blood clotting system. The decarboxylated coagulation factors produced in the presence of warfarin are bio-

logically inactive and successively replace the active clotting factors in the circulatory system, explaining the slow onset of the anticoagulant effect of warfarin. Warfarin and related drugs are referred to as vitamin K antagonists, and their effects can be reversed with vitamin K or by transfusion of coagulation factors. The inter- and intraindividual therapeutic sensitivity to warfarin is variable and the intensity of the anticoagulant effect must be followed with standardized measurements of the prothrombin time (international normalized ratio, INR).

1.9 Neurochemical markers of brain damage

Measurement of concentrations of organ-specific proteins in body fluids is well established in various medical fields, e.g. analysis of serum levels of troponin and creatine kinase in patients with suspected myocardial infarction or alanine aminotransferase to evaluate liver cell damage. Specific proteins that are found in high concentrations in CNS but in negligible concentrations in other organs can be used to assess various types of brain injury. The neurochemical marker proteins are specific for different CNS cell types and/or for distinct cellular components. Of the two brain damage markers used in this thesis (Paper I) the light chain of the neurofilament protein (NFL) is an established marker of neuronal/axonal damage, and glial fibrillary protein (GFAP) is a marker of astroglial cell injury. Ischemic infarctions and other types of acute or chronic brain diseases provoke brain cell destruction and leakage of marker proteins into CSF. NFL and GFAP are analyzed in clinical praxis at the Sahlgrenska University Hospital.

The neurofilament is the main structural component of the neuronal cytoskeleton. It is abundant in axons relative to nerve cell somas and in large myelinated axons (129). The neurofilament is composed of a triplet protein of which the light subunit (NFL) is the essential component. It is used as a marker of neurodegeneration, particularly of axonal damage. Following acute parenchymatous CNS damage, the levels gradually increase, with a maximum after a few weeks and normalization over a period of months. The late increase is related to the release of NFL from injured axons subsequent to damage of neuronal soma. Measurements of CSF-NFL as a marker of axonal damage show increased values after cerebral infarction (130-132) and in various other conditions with neuronal damage (131, 133-135).

Glial fibrillary acidic protein is a structural protein found almost exclusively in glial cells of the CNS. In astrocytes, GFAP builds up the intermediate filament, which is the main cytoskeleton structure (136). Increased levels of GFAP in CSF have been demonstrated in two principally different situations: (i) associated to astrogliosis and (ii) after acute CNS injuries with dis-

integration of glial cells. Astrogliosis is a reactive proliferation, and hypertrophy of astrocytes with increased intracellular amounts and extracellular release of GFAP to CSF. Astrogliosis is an unspecific reaction seen following brain injury, in inflammatory and certain chronic conditions, but also during ageing (137). Modest increases in CSF-GFAP levels are seen in multiple sclerosis (133) and normal pressure hydrocephalus (138). After acute parenchymatous CNS damage, an increase in CSF-GFAP can be detected from days 1-2, with concentrations returning to normal within less than three weeks (139). The levels reflect the extent of the injury and are high after large cerebral infarctions (139, 140), in patients with herpes encephalitis (134) and after subarachnoid hemorrhage (141).

2 Aims of the study

1. To study the incidence of symptomatic and silent cerebral complications during episodes of left-sided infective endocarditis using sensitive diagnostic methods (Paper I).

2. To study the influence of protective factors and risk factors for cerebrovascular complications in left-sided infective endocarditis (Paper I-III).

3. To study the relationship between previously established daily antiplatelet therapy and cerebrovascular complications and mortality in left-sided definite infective endocarditis (Paper II).

4. To study the association between ongoing oral anticoagulant therapy and cerebrovascular complications in patients with left-sided native valve endocarditis (Paper III).

3 Patients and methods

3.1 Patients

Adult patients with left-sided possible or definite IE according to the Duke modified criteria (6) at three tertiary care centers in Sweden and Denmark were included. Altogether, 866 episodes of IE in 837 patients were consecutively enrolled in the study from January 1, 1996 to January 31, 2008 in Gothenburg (papers I–III) and from October 1, 2002 to January 31, 2008 in Copenhagen (papers II–III). Patients with isolated right-sided IE were excluded from the analysis owing to the inherently low risk of cerebral embolism in such episodes (68). Thus the remaining 797 episodes of left-sided IE in 771 patients were the total study cohort as illustrated in figure 2**.** In paper I, only patients from Sweden were included, and the inclusion period was in two intervals between June 1, 1998 and January 31, 2005. During the final year of that study, patients from the Department of Infectious Diseases at Skaraborg Hospital, Skövde, Sweden were also included.

The Department of Infectious Diseases, Sahlgrenska University Hospital, Gothenburg, Sweden, serves the Gothenburg area, with about 600 000 inhabitants, as both first-line and tertiary care center for IE patients. In addition, the Departments of Infectious Diseases and Cardiothoracic Surgery at the Sahlgrenska University Hospital serve as tertiary referral center for IE patients from other hospitals in western Sweden (Västra Götaland regionen) with about 1.5 million inhabitants. The Department of Cardiology, Gentofte University Hospital, Copenhagen, Denmark and the Department of Cardiology, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark provide care for patients with IE in the eastern part of Denmark with a catchment area of 2.4 million inhabitants. These centers are the only ones in the area receiving IE patients, and are thus responsible for both basic and highly specialized care. Patients with diagnosed IE are transferred to these centers and there are cardiothoracic surgery departments in both hospitals.

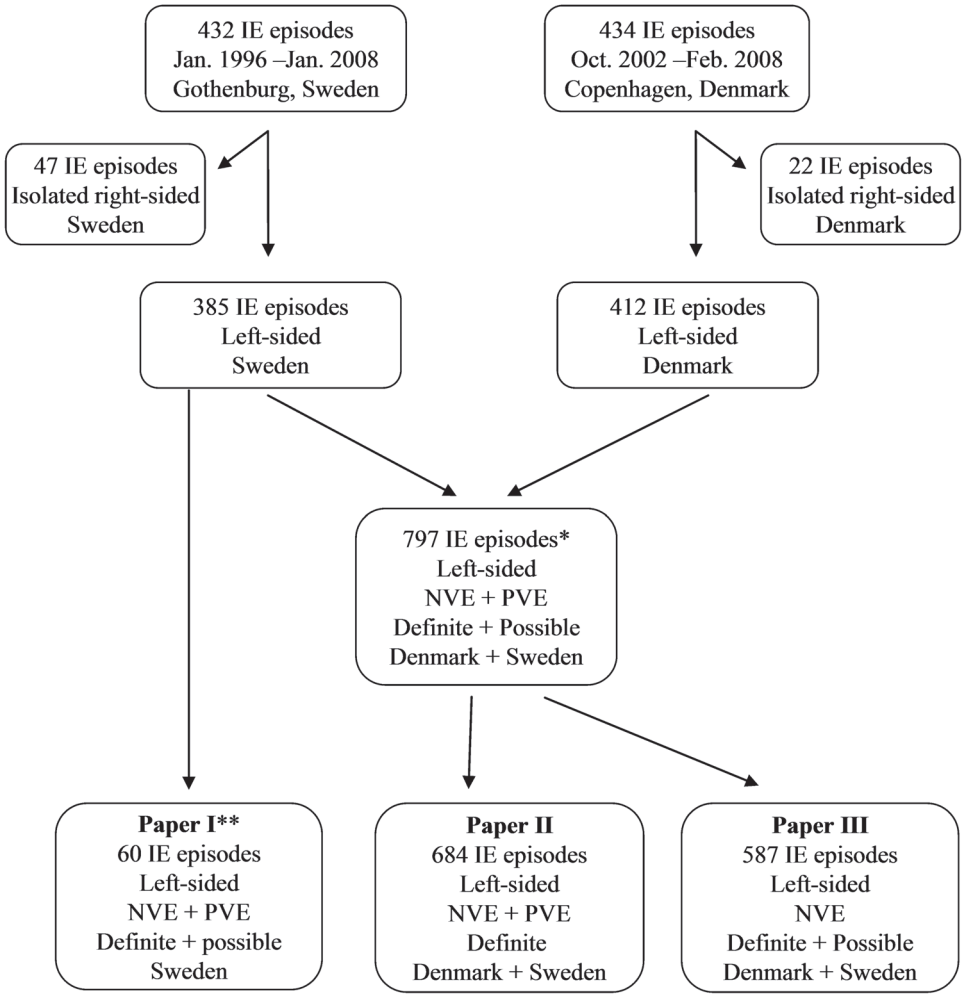

Figure 2. Distribution of patients with infective endocarditis included in the studies. IE: infective endocarditis, NVE: native valve endocarditis, PVE: prosthetic valve endocarditis.
** Referred to in text as "total cohort".*
*** Inclusion period: June 1998-April 2001, September 2002-January 2005. Two patients from the Department of Infectious Diseases at Skaraborg Hospital, Skövde, Sweden included in 2004.*
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3.2. Study design

The studies were carried out as observational cohort studies. In paper I, patients with high clinical suspicion of IE were enrolled irrespective of neurological symptoms. Symptomatic and silent (asymptomatic) CVC during the

present IE episode was the primary outcome variable. Patients underwent repeated physical and neurological examinations during hospitalization. Magnetic resonance imaging (MRI) of the brain was performed during the first 10 days of antibiotic treatment and a follow-up MRI after 2-3 months. Lumbar puncture with analysis of CSF was done during the first and fourth weeks of treatment if no contraindications were found and the patient consented. The study was approved by the ethics committee of the University of Gothenburg (L 077-98).

In papers II–III consecutive patients with IE treated in the participating centers were eligible for study entry. Patients enrolled prospectively in the study were followed according to local protocols including uniform treatment regimens, clinical evaluation procedures and collections of specimens including blood cultures. Study participants were treated in regular care at the Cardiology Department (Denmark) or the Infectious Diseases Department (Sweden) and consultations with other specialists including thoracic surgeons were performed regularly. Demographic and clinical characteristics including age, sex, presence of comorbidities, ongoing medication, cardiac rhythm, presence of prosthetic valve, or pacemaker/intravascular device and duration of symptoms prior to admission were recorded. Blood cultures, biochemical blood tests, radiological, electrocardiographic and echocardiographic examinations as well as antibiotic treatment, and frequency, timing and type of surgical treatment were recorded. Primary outcome variables were cerebrovascular complications (CVC) during IE, and in-hospital and 12-month mortality.

3.3 Methods

Magnetic resonance imaging (Paper I)

The MRI protocol contained T1, T2, and PD-weighted images before and after the administration of a gadolinium contrast medium. MR angiography was not routinely performed. Diffusion-weighted MRI was not available when the study began and is therefor not included in the protocol. If contraindications for MRI were present, computed tomography (CT) of the brain with and without contrast was performed instead. MRI findings were evaluated directly by the supervising neuroradiologist and later reevaluated by one of two experienced neuroradiologists blinded to the clinical and neurological status of the patients. Identification of acute or subacute ischemic, hemorrhagic or infectious lesions as well as changes between the two subsequent neuroradiological investigations in each patient were considered in the diagnosis of a cerebral complication. Each patient served as his/her own control,

and findings with a temporally consistent association were considered related to the present IE episode.

Cerebrospinal fluid analyses (Paper I)

The CSF analyses included bacterial culture, cell counts, and determination of glucose and albumin levels. Polymorphnuclear leukocytes >5 x 10^6 cells/mL CSF were considered diagnostic of meningitis (49, 54), while isolated elevation of monomorphnuclear leukocytes was not.

CSF concentrations of the neurochemical brain damage marker NFL (neurofilament protein light chain) were analyzed using a sandwich ELISA as previously described (131). The sensitivity of the assay was 125 ng/L and the standard curve ranged from 125 to 16 000 ng/L. Values are age dependent and were regarded as normal if the level of CSF-NFL was <250 ng/L for patients aged <59 years, <380 ng/L for patients aged 60-69 years and <750 ng/L for patients aged >70 years.

GFAP (glial fibrillary acidic protein) was measured with a previously described ELISA procedure (137). The standard curve ranged from 32-16 000 ng/L and the sensitivity of the assay was 16 ng/L. Values are age dependent and were regarded as normal if the level of CSF-GFAP was <750 ng/L for patients aged 20-59 years, <1250 ng/L for patients aged >60 years. CSF-NFL and CSF-GFAP reference levels were based on measurments from 141 neurologically healthy individuals aged 18-83 years.

Echocardiography

Transthoracic and transoesophageal echocardiography were performed using standard techniques in all patients included in papers I-III with the exception of 17 cases where only TTE was performed. Seven of these were classified as possible IE due to lack of conclusive findings on TTE, four were definite through autopsy findings, one through peroperative findings, four through detection of vegetations on TTE, and one classified as definite based on five minor criteria.

Evidence of endocardial involvement detected using echocardiography was classified according to the modified Duke criteria (6). Vegetations were classified by location and maximum size. Mobility characteristics were considered as an insufficiently standardized variable in the absence of systematic reevaluation of the echocardiographic examinations and were therefore not included in any of the analyses. Paravalvular engagement of the infection was defined as the presence of intracardiac abscesses and/or pseodoaneurysm formation or new periprosthetic regurgitation in PVE.

3.4 Definitions

Case definition

A prospectively identified episode of left-sided IE satisfying the modified Duke criteria (6) of possible or definite IE constituted a defined case. Possible IE episodes were included if treatment was given in accordance with IE treatment guidelines or, in cases of in-hospital death, intended to be given. In cases included prior to the year 2000 the modified criteria were applied retrospectively. Childhood cases were not included (<15 years). Left-sided IE cases included episodes with combined right and left-sided engagement of the infection.

Microbial etiology

The causative agents were divided into seven different categories of microbial etiology: *Staphylococcus aureus* including methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains; viridans group streptococci including *Streptococcus bovis;* coagulase-negative staphylococci; *Enterococci* spp; other streptococci including groups A, B, C, G and pneumococci, miscellaneous microorganisms including HACEK (*Haemophilus* spp, *Aggregatibacter* [former *Actinobacillus*] *actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella* spp), *Candida spp* and other bacteria; and episodes with negative blood cultures.

Cerebrovascular complications

Cerebrovascular complications (CVC) included ischemic and hemorrhagic infarctions, intracerebral and subarachnoid hemorrhages, cerebral mycotic aneurysms, transient ischemic attacks (TIA), and cerebral infections including brain abscesses and culture-positive and culture-negative meningitis. A TIA was defined as a focal neurological sign or symptom of sudden occurrence and resolution within 24 h.

Cerebral events occurring in the prediagnostic period and during treatment of the studied IE episode were the focus of this thesis, while later neurological complications were not systematically registered. Diagnosis of CVC was based on repeated physical and neurological examinations, neuroimaging recordings and/or lumbar puncture. Cerebral CT or MRI was performed in all episodes with neurological symptoms except six cases in the total study cohort. In five cases presenting with meningism, only lumbar puncture with CSF analysis was performed and in one case of early in-hospital death owing to cardiac failure, a clinical diagnosis of ischemic stroke was made. In patients without neurological symptoms no routine cerebral CT or MRI was performed.

Mortality

In-hospital mortality was defined as death occurring during the index hospitalization for IE. Twelve-month mortality (paper II) included death occurring during the initial hospitalization and the follow-up period.

Antiplatelet therapy

Antiplatelet therapy (paper II) included acetylsalicylic acid (ASA), dipyramidole or clopidogrel, or combinations of these agents. Doses were recorded. No patients were treated with ticlopidine. Previously established use was defined as prescribed and reported daily use of an antiplatelet agent prior to admission to hospital.

Anticoagulant therapy

Ongoing oral anticoagulant therapy (paper III) was defined as prescribed and reported continuous use of an oral anticoagulant prior to admission for IE. The only oral anticoagulant agent used by study patients was warfarin. Discontinuation of warfarin was registered, as was replacement therapy with heparin or low molecular weight heparin.

3.5 Statistics

Comparisons of continuous data were performed using Mann-Whitney U test, student´s *t* test or one-way ANOVA when accurate. For categorical variables the Chi-square test or Fisher's exact test was used. Continuous variables were expressed as median and interquartile range (IQR) or mean and standard deviation. All statistical tests were two-tailed and p-values less than 0.05 considered statistically significant. Univariable risk factor analysis was performed using Fisher's permutation test (142) in paper I and univariable logistic regression in papers II and III. Multivariable risk factor analysis was performed using logistic regression models in papers I-III. The multivariable models contained traditional risk factors, such as age and sex, as well as factors statistically significant in the univariable models in each study. In paper I the discriminating ability of variables in the multivariable model was tested using receiver operating characteristic (ROC) curves. The area under the ROC curve equals the probability that a randomly chosen individual with a certain risk factor will have a higher value of the discriminating variable than a randomly chosen individual without this risk factor. The analysis is considered to have excellent discrimination if the area under the ROC curve is in the interval 0.8-0.9 (143).

4 Results and discussion

4.1 Demography

Baseline characteristics of the 797 episodes of left-sided IE in the total study cohort are shown in table 2. Infective endocarditis fulfilling definite criteria was seen in 86% of the episodes. The prevalence of intravenous drug use (IVDU) was generally low among study patients but, as expected, a higher rate of IVDU was reported among patients with *S. aureus* IE compared to patients with IE caused by other pathogens (7% vs. 2%, p=0.007). Prosthetic valve endocarditis was seen in 26% of the patients and a history of congestive heart failure in 23%. Other underlying conditions such as diabetes (22% vs. 10%, p<0.001), immunosuppression (15% vs. 9%, p=0.026) and hemodialysis (9% vs. 2%, p<0.001) were more prevalent in patients with *S. aureus* IE. Cardiac surgery during antibiotic treatment was performed in 39% of the patients in the total cohort.

Age

Mean and median age of patients in the total cohort was 63.5 (SD 15.9) and 66 (IQR 53-76) years, with a range of 15-99 years. A similar age span was seen in the National Swedish Endocarditis Registry (57, 144) and in other modern IE cohorts in the industrialized world (24, 31, 118, 145), while the median age in patients in a large worldwide multicenter study performed by the International Collaboration on Endocarditis (ICE) was slightly lower (42). These aged populations contrast the IE cohorts studied in the mid-1900s (45, 146) and in developing countries today (147, 148) with mean ages of 40 years or less. The numbers of patients divided by 10-year age intervals are shown in figure 3. The number of IE episodes increased in each 10-year age group up to 80 years of age, but no incidence rates were calculated owing to lack of denominator data. However, in a study from Gothenburg 1984-1988 also including retrospectively identified cases (19) median age was still higher, 70 years, and the calculated incidence continued to increase beyond 80 years of age.

Median age (67 years) among patients included in paper I was in accordance with the median age of the total cohort despite the different inclusion procedure in that part of the study. Among definite IE cases (paper II), the subgroup of patients on previously established antiplatelet therapy had a median

Table 2. Demographic and clinical characteristics of 797 left-sided infective endocarditis episodes.

	Study patients, left-sided IE episodes n=797 (%)
Definite infective endocarditis	684 (86)
Female sex	264 (33)
Age (years) median (IQR)	66 (53-76)
Diabetes mellitus	100 (13)
Hemodialysis	28 (4)
History of congestive heart failure	180 (23)
Previous infective endocarditis	76 (10)
Atrial fibrillation	143 (18)
Intravenous drug use	27 (3)
History of malignancy	61 (8)
Immunosuppression	83 (10)
Aortic valve infection	498 (62)
Mitral valve infection	396 (50)
Dual valve infection	97 (12)
Prosthetic valve infection	210 (26)
CRP maximum level (mg/L) median (IQR)	117 (65-200)
Duration of antibiotic therapy (days) median (IQR)	32 (27-42)
Cardiac surgery during antibiotic treatment	312 (39)

IQR: interquartile range, CRP: C-reactive protein

age of 74 years, as compared with 62 years (p=0.001) among patients without antiplatelet therapy. Since specific diseases and medical conditions associated with indications for antiplatelet therapy have an age-dependent prevalence this was an expected finding and is in agreement with previous studies dealing with chronic antiplatelet therapy in IE (51, 53, 118, 119). In paper III, where only episodes of NVE were studied, patients on warfarin tended to

be older than patients not on warfarin, but the differences did not reach statistical significance for median ages (69 vs. 64 years, p=0.058). Patients with PVE were significantly older than patients with NVE (71 vs. 64 years, p<0.001)

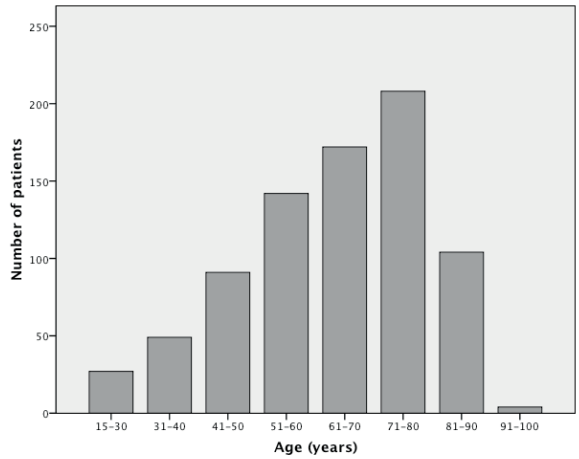

Figure 3. Number of patients with infective endocarditis divided by 10-year age intervals. Total number of episodes 797.

Sex differences

In the total cohort of 797 IE episodes, 33% (264) were seen in women, Women were, on average, older than men (68 vs. 65 years, p=0.02). The proportion of definite IE did not differ significantly between the sexes. Mitral valve involvement was more common in women than in men (54 vs. 46%, p=0.029) but the presence and length of vegetations did not differ between the sexes. Contrary to a study concerning sex differences in IE by Aksoy et al. (50) there was no statistically significant difference between the frequency of comorbid conditions between women and men. A higher proportion of IE episodes in women were caused by *S. aureus* as compared with the proportion in men (29% vs. 20%, p=0.003).

In the total cohort, CVC were seen in 26% of the female episodes as compared with 20% of the male episodes (p=0.06). When definite episodes were studied separately (paper II), the higher CVC frequency in women was statistically significant (30% vs. 22%, p=0.039). However, when the patients were stratified for microbial etiology, the CVC frequency did not differ between the sexes. This is contrasted by the findings in a study of 260 *S. aureus* IE by Roder et al. in which women tended to have more neurological

complications than men (55). Among episodes of definite IE, cardiac surgery during hospital stay was performed in 40% of the women vs. 44% of the men (n.s.). There was no statistically significant difference in in-hospital mortality between women and men (17% vs. 13%, n.s.), but 12-month mortality was higher in women (31% vs. 24%, p=0.031), similar to the findings of Thuny et al. (58).

4.2 Microbiology

The most commonly found pathogens in the total cohort of 797 patients were viridans group streptococci (including *S. bovis*) seen in 29% of the episodes (figure 4). *S. aureus* was the etiological agent in 23% of all cases and in two episodes MRSA strains were identified. In paper II, where definite IE cases were studied separately, the *S. aureus* etiological fraction was 25% (168/684). This is consistent with the 28% *S. aureus* IE among 1213 definite IE cases from European countries prospectively included from 2000-2005 in the International Collaboration on Endocarditis (ICE) study (42). In paper I, the proportion of *S. aureus* IE among study patients was 25%.

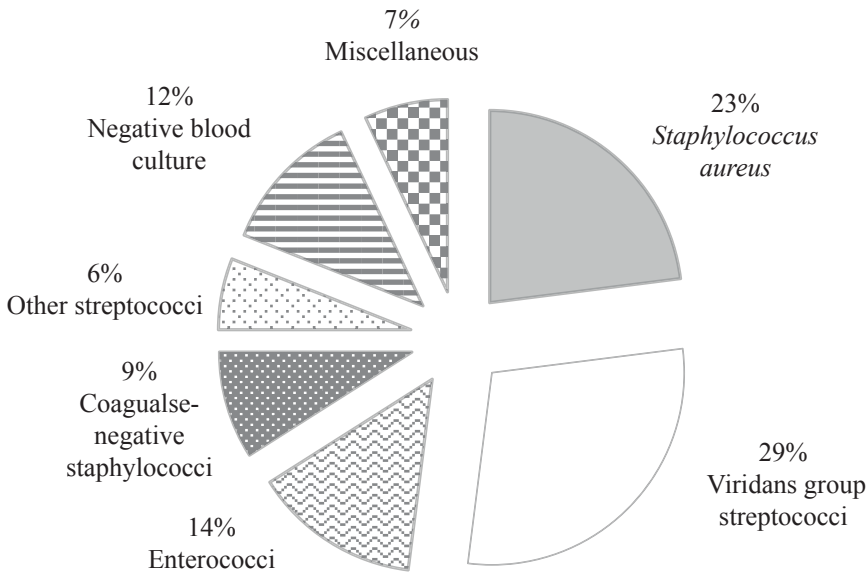

Figure 4. Microbiological etiology of 797 left-sided infective endocarditis episodes.

Negative blood cultures were seen in 12% of all episodes, and 58% of the blood culture negative episodes were classified as possible. This is compatible with an analysis of culture negative IE reported in the National Swedish Endocarditis registry from 1995-2004, where the proportion of culture negative episodes was 12%, and 75% were classified as possible IE (57). In a prospective epidemiological study from Gothenburg and Borås, Sweden (1984-1996), the proportion of culture negative IE was higher, 20%, and only one fifth of the culture negative IE cases were classified as definite IE (149). The combined secondary and tertiary referral center characteristics of the including sites in our study probably conferred a selection bias affecting the proportion of blood culture negative IE episodes.

Patients with enterococcal IE were significantly older (median 72 years) than patients with IE of other etiologies (median 60-65 years, p=0.01) except for coagulase-negative staphylococci (median 68 years), where the difference was not significant (figure 5). Enterococcal IE has been more prevalent in studies specifically concerning IE in older patients (41, 150). Prosthetic valve endocarditis (n=210) were caused by coagulase-negative staphylococci in 17% of the cases, in another 17% of the cases by viridans group streptococci, and in 19% each by *S. aureus* and enterococci. Of coagulase-negative staphylococcal IE 51% were PVE as compared with 24% PVE episodes with other pathogens (p<0.001).

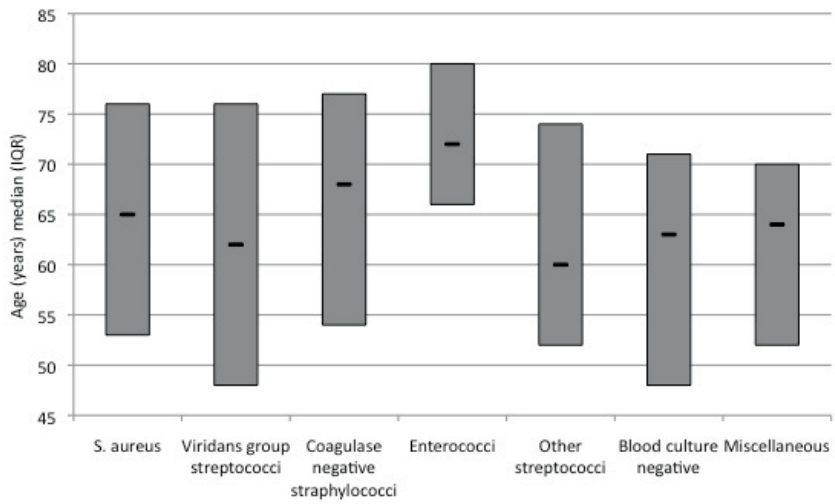

Figure 5. Median age (interquartile range) among 797 IE episodes divided by pathogen.

4.3 Cerebrovascular complications

Symptomatic cerebrovascular complications

Cerebrovascular complications with concomitant neurological symptoms were diagnosed in 22% (177) of the IE episodes in the total cohort. The incidence of CVC in papers II and III was 25% in each. This higher figure is explained by the fact that CVC were diagnosed more often during definite IE episodes (paper II) as compared with possible episodes (25% vs. 7%, $p<0.001$), and that significantly more CVC were detected in episodes of NVE as compared with PVE (24% vs. 16%, $p=0.008$). Median ages did not differ significantly between patients with and without CVC. The CVCs were divided into three major groups; ischemic (including both established infarctions and TIA), hemorrhagic, and infectious complications depending on the type of sign or lesion presented. The proportion of episodes where two or more types of cerebral lesions were detected was 4% in the total cohort. More than one neurological symptom was displayed by 15% of the 60 patients in paper I, although one of the symptoms dominated in each patient's initial presentation. Neurological symptoms were present on admission for IE in three quarters of the patients with symptomatic CVC (paper I: 76%, paper II: 73%, paper III: 74%). Totally 17% of all patients experienced a CVC during admission (half recurrent CVC, half first time CVC) in paper I compared to 8% in papers II-III. The frequency of patients who underwent cardiac surgery after hosiptal admission for IE did not differ between patients with and without CVC in the total cohort (42% vs. 38%, n.s.).

Neurological symptoms were displayed by a significantly higher proportion of the patients in paper I (35%) than in papers II-III (25%, $p=0.003$). The high proportion of patients found to have symptomatic cerebral complications in paper I could be explained by the additional clinical diagnostic efforts in the study with repeatedly performed standardized neurological examinations of the patients included, apart from the radiological and neurochemical investigations also undertaken. A higher diagnostic sensitivity of this procedure could be assumed as compared with the routine physical and neurological examinations performed in patients in the other parts of the study.

In paper I patients were not included consecutively owing to late transfer of a considerable proportion of IE patients from other hospitals, the possible inclusion period of the study thereby being passed. Some patients were also reluctant to participate in a study involving additional investigations in the early period of this often unexpected and serious disease. The non-consecutive inclusion of IE patients might have impacted on the number of

patients with neurological symptoms included, but no selection of neurologically symptomatic IE patients was done during the study period.

Ischemic infarctions and TIA

Ischemic infarction was the most common type of CVC, seen in 15% (120/797) of the episodes in the total study cohort. In paper I patients with neurological symptoms had ischemic lesions in 18 episodes, corresponding to 30% of the 60 patients included. Infarctions among the neurologically symptomatic patients were verified using MRI and release of brain damage markers in 11 cases, only by MRI findings in six cases and by isolated elevation of NFL and GFAP in one case. There were multiple ischemic lesions on MRI in 10 patients, ranging from three to more than 10 in each patient. The sizes varied from punctuate to 3 cm, and in one patient there was a more specific pattern of septic embolism with early stages of abscess formation. The lesions were distributed in various parts of the brain including the cortical, subcortical, cerebellar and thalamic regions.

In paper III, where 587 NVE episodes were studied separately, 96 cerebral ischemic infarctions verified by CT or MRI were found out of 144 episodes with symptomatic CVC (67%) (table 3). The size and localizations of these infarctions were not recorded systematically in the total study cohort, but all patients were neurologically symptomatic and the majority displayed focal or multifocal neurological symptoms.

Table 3. Types of cerebral lesions in 144 native valve endocarditis episodes with cerebrovascular complications (paper II).

	Number of NVE episodes with CVC n=144	Proportion of all NVE episodes n=587
Ischemic infarction	96 (67%)	16%
Transient ischemic attack	20 (14%)	3%
Hemorrhagic lesion	14 (10%)	2%
Ruptured mycotic aneurysm	2 (1%)	0.3%
Cerebral infection	38 (26%)	6%
More than one type of cerebral lesion	26 (18%)	4%

Among the 282 Swedish patients with NVE included in paper III, 16% suffered a symptomatic ischemic infarction verified by CT or MRI. Twenty percent of these 44 patients died during index hospitalization, 18% had major sequelae (hemiparesis, aphasia) and 25% minor sequelae (minor weakness, dysphasia, cognitive impairment) at hospital discharge. Studies on neu-

rological recovery in IE patients are scarce but our figures can be compared with the findings of Ruttmann et al. (52) in a study of 214 surgically treated IE patients. Thirty percent of the patients in that study had cerebral complications prior to cardiac surgery. Overall 54% of the patients with preoperative cerebral lesions achieved full neurological recovery (in-hospital mortality 17%), but a worse prognosis was seen in patients with large cerebral infarctions and patients with multiple types of neurological complications. Stroke in IE patients was concluded to have a favorable prognosis as compared with stroke resulting from other causes.

A TIA was diagnosed in 3% of the 587 NVE episodes in paper III and in one out of 60 IE episodes (2%) in paper I. Similar incidences of TIA were found by Heiro et al. (5%) at a Finnish teaching hospital with IE patients included from 1980-1996 (49), and in a study by Thuny et al. (6%) among 496 prospectively studied definite left-sided IE episodes 1990-2005 at two French referral centers (72). Since a TIA, by definition, only gives transient neurological signs, a risk for underdiagnosis accompanies this condition, but the specificity in the diagnosis of TIA could also be questioned.

Cerebral hemorrhage

Cerebral bleeding was detected in 14 out of the 587 NVE episodes (2%) and in two additional patients with PVE, the frequency of cererbral hemorrhage in PVE thereby being 1% (2/210). Accordingly, in 16 of the 797 episodes in the total cohort (2%) some degree of cerebral bleeding was found, and 75% of these complications had already occurred before admission to hospital. The cerebral hemorrhage was characterized as primary intracerebral in seven cases, caused by a ruptured mycotic aneurysm in two cases, complicating a primary infarction in six cases, and accompanying a brain abscess in one case. Patients suffering from cerebral hemorrhagic complications in IE were younger than patients with other types of CVC (median 54 vs. 65 years, p=0.024). The causative pathogen was *S. aureus* in 56% (9/16) of the IE cases with a hemorrhagic complication as compared with 49% (59/120) *S. aureus* IE among patients with ischemic infarctions (n.s.). In-hospital mortality among patients with intracerebral bleeding complications was 38% (6/16), not significantly different from the in-hospital mortality of 25% (41/161) seen in patients with other types of CVC. In paper I the incidence of symptomatic cerebral bleeding was 2% (one patient) while minor hemorrhagic components not influencing the management of the patients were found in another five patients.

Mycotic aneurysms were detected in three patients in the total cohort, two of whom presented with a subarachnoid hemorrhage on admission. In the third patient, MRI detected an aneurysm without signs of bleeding that healed during antibiotic therapy. Blood cultures were negative in two cases and

grew *Cardiobacterium hominis* in the third case. None of the patients with mycotic aneurysms died during admission. One of the patients, with a ruptured mycotic aneurysm on admission, underwent cardiac surgery on treatment day 71 owing to progressive aortic insufficiency. Two intracerebral mycotic aneurysms had been coiled prior to the cardiac operation. An additional patient presented with a ruptured mycotic aneurysm four months after the completion of antibiotic therapy for aortic NVE caused by *Salmonella enteritidis*. This illustrates a well-known risk of late cerebral complications in IE (47, 151).

Meningitis and brain abscess

Signs of cerebral infection were found in 40 out of 797 patients (5%) in the total cohort, and in 20 there were other concomitant neurological signs. Cerebral CT diagnosed a brain abscess in three patients and the remaining 37 were characterized as episodes of meningitis by findings in lumbar puncture. The CSF pleocytosis was usually moderate and ranged from 11 to 3 000 x 10^6 white blood cells/mL with a median of 99 (IQR 25-134) x 10^6 white blood cells/mL. In two cases culture of CSF was positive, but most lumbar punctures were performed after the institution of antibiotics. In one case, CSF culture grew *S. aureus* (polymorphnuclear leucocytes 96 x 10^6/mL) and in one case *Streptococcus oralis* (polymorphnuclear leucocytes 3 000 x 10^6/mL). In the total cohort lumbar puncture was only performed in patients with clinical suspicion of meningitis or occasionally on the basis of clinical suspicion of subarachnoid bleeding (one case with sudden severe pain in the occipital part of the head), and when no contraindications for this investigation were perceived. CSF-NFL and CSF-GFAP were not used to detect cerebral parenchymatous involvment in papers II and III. A normal CSF finding was only seen in four patients, probably indicating a low tendency to perform lumbar puncture in this type of patients. In-hospital mortality in patients with meningitis as the only cerebral complication was 11% (2/18) and one additional patient had minor sequelae at hospital discharge. In paper I isolated meningitis was found in two out of 60 patients (3%).

Encephalopathy

The clinical entity referred to as encephalopathy in earlier series of IE (54, 55, 77, 82, 152) has been documented clinically in our study cohort as patients presenting with a mixed picture of altered consciousness, variable degree of meningism and minor focal neurological signs on neurological examination, but the term encephalopathy is not used in papers II and III. The inclination to perform neuroradiological examinations, e.g. CT scan of the brain, to further characterize various neurological symptoms in IE patients was high during the study period. The number of cerebral events thereby classified as infarctions instead of encephalopathy was probably higher in our study than in studies including patients from the 1980s (49, 54,

77) when CT examinations assumedly were less common. In still earlier studies, such as the study by Pruitt et al. published in 1978 (47), the high in-hospital mortality rates in IE patients with neurological complications (58%) and the considerable proportion of post-mortem examinations executed in these patients (>90%) facilitated more precise diagnosis. The detected incidence of major cerebral infarctions was 17% in that study, and an additional 11% of the patients were found to have microembolic ischemic lesions. Patients with microembolic lesions presented with altered levels of consciousness and fluctuating neurological signs, in accord with the clinical description of encephalopathy.

In paper I a more thorough characterization of patients with encephalopathic neurological manifestations was possible, and all these patients presented with confusion and/or altered consciousness and minor focal signs. Altogether six out of 60 patients (10%) were considered to be encephalopathic, and the median age of these patients was 78 years as compared with 54 years in patients presenting with other neurological signs (n.s.). In all six patients with the clinical picture of encephalopthy there were multiple ischemic lesions demonstrated on MRI, and increased levels of GFAP and NFL were detected in CSF, illustrating the embolic nature of these lesions and the involvement of parenchymatous CNS damage.

4.4 Factors related to the incidence of cerebrovascular complications

Antiplatelet therapy (paper II)

In paper II the aim was to study the relationship between previously established antiplatelet therapy and the incidence of cerebrovascular complications in left-sided definite IE episodes. Antiplatelet therapy was established prior to IE diagnosis in 23% of the 684 IE episodes in patients included in this part of the study. No difference in the incidence of CVC during IE was detected between patients with and without previously established antiplatelet therapy (24% vs. 25%, OR 0.9, 95% CI 0.6-1.4). To adjust for possible confounding variables a multiple logistic regression model was used, and variables that were significantly associated with both occurrence of CVC and to the use of antiplatelet therapy were included. After adjustment for congestive heart failure, vegetation length, pathogen and CRP level no significant relation between antiplatelet therapy and CVC incidence was found either (aOR 0.8, 95% CI 0.48-1.5).

The possibility of a reduced embolic risk owing to use of antiplatelet therapy during the prediagnostic development of IE has been debated (51, 53, 153,

154) while in a prospective study, the initiation of antiplatelet therapy after IE diagnosis was found not to reduce the embolic risk (117). A study on specific influence of antiplatelet therapy on mortality rate (118) and of ASA on the rate of acute valvular replacement surgery in *S. aureus* IE (119) has been published recently. Only one small previous study has focused on the role of antiplatelet therapy in relation to cerebral complications (155).

Anticoagulant therapy in native valve endocarditis (paper III)

In paper III, including episodes of NVE, 8% (48/587) of the patients were on oral anticoagulants (warfarin) upon admission. There was, as expected, a significantly higher prevalence of atrial flutter among patients on warfarin as compared with patients not on warfarin (37% vs. 12%, p<0.001). Surgical rates did not differ between patients with and without warfarin on admission for IE.

Symptomatic CVC were significantly less frequent in NVE patients on warfarin than in patients not on this treatment, i.e. in 6% vs. 26% (OR 0.2, 95% CI 0.06-0.6, P=0.006). In the warfarin group all CVC were established on admission, while 7% of the patients without warfarin experienced a first CVC during antibiotic treatment. The reduction in CVC rate consisted of a lower number of non-hemorrhagic events, while the incidence of hemorrhagic complications did not differ significantly between patients with and without warfarin therapy (figure 6). After adjustment for age, sex, *S. aureus* etiology, history of congestive heart failure or previous IE episode, vegetation length and CRP level, a significantly lower incidence of CVC prevailed among patients on warfarin (aOR 0.26, 95% CI 0.07-0.94, p=0.04).

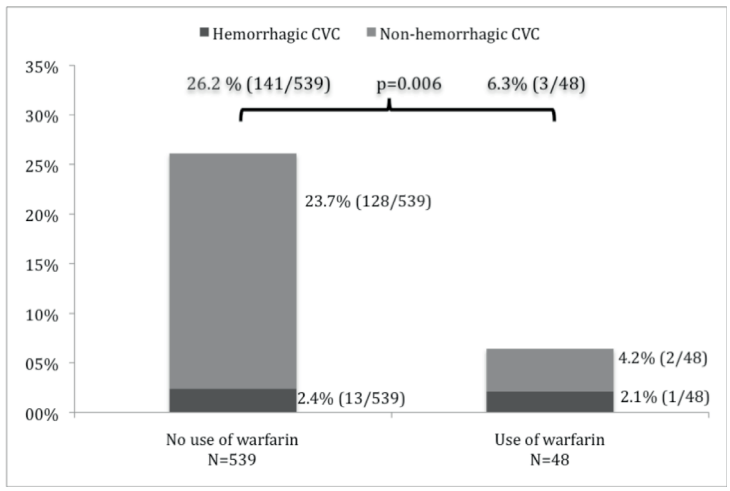

Figure 6. Cerebrovascular complications in 587 left-sided native valve endocarditis episodes in patients with and without warfarin therapy

The aim in paper III was to do a specific analysis of the relation between ongoing therapy with vitamin K antagonists and the incidence of CVC in IE affecting native valves. This design was chosen in order to avoid confounding from inherent differences in (i) CVC rate between NVE and PVE, (ii) PVE affecting different types of valve prostheses (mechanical, bioprosthetic, homograft) and (iii) effects on CVC rate between oral anticoagulants and heparin analogues.

Anticoagulant therapy and prosthetic valve endocarditis

In the total cohort of 797 patients there were 210 PVE not separately analyzed in papers I-III. As earlier mentioned, the CVC incidence was lower among PVE cases than in NVE (16% vs. 24%, p=0.008). The incidence tended to be lower in bioprosthetic PVE (13%, 13/98) than in mechanical PVE (18%, 20/112) but this difference was not statistically significant. Warfarin use on admission for PVE was frequent (67%), and, as expected, it was more frequent in PVE on mechanical valve prostheses compared to PVE on bioprostheses (90%, vs. 41%, p<0.001). CVC incidence in PVE did not differ between patients with and without warfarin therapy (17% [24/141 vs. 13% [9/69], n.s.), and this was similar in both mechanical and biological PVE. These findings evoke the hypothesis that embolic events in PVE and NVE have different predictors and favor separate analysis of the disease entities. Some recent studies have found a trend towards fewer CVC in PVE compared to NVE (49, 55, 65) or a lower rate of systemic major embolism in PVE (156), while the incidence of cerebral complications in NVE and PVE did not differ in other studies (48, 54, 72).

Microbial etiology and CVC

Patients with *S. aureus* IE suffered from cerebral complications significantly more frequently than patients with IE caused by other pathogens. The CVC incidence in *S. aureus* IE was 49% (89/182) in the total cohort, which corresponded to 52% among both definite IE episodes (paper II) and NVE episodes (paper III). In table 4 unadjusted OR for CVC divided by pathogen in definite IE episodes are shown (paper II). Enterococcal IE carried the lowest CVC risk, significantly different from the risk seen in culture-negative IE (OR 2.8, 95% CI 1.1–7.0) and *S. aureus* IE (OR 8.5, 95% CI 4.4-16.8). In both papers I and III, *S. aureus* etiology remained a significant risk factor for symptomatic CVC in the multivariable models, with an adjusted odds ratio of 6.1 (95% CI 1.5-24.3) and 6.3 (95% CI 3.8-10.4), respectively.

IE episodes complicated with meningitis had a different etiological pattern, since this manifestation was seen more often in IE caused by non-viridans

Table 4. Odds ratio for cerebrovascular complications divided by pathogen in 684 definite infective endocarditis episodes.

Pathogen	***% CVC***	***OR***	***95% CI for OR***
Enterococci (n=108)	11% (n=12)	reference	-
Coagulase-negative staphylococci (n=59)	14% (n=8)	1.3	0.5 - 3.3
Viridans streptococci (n=214)	15% (33)	1.5	0.7 – 2.9
Other streptococci (n=47)	19% (n=9)	1.9	0.7 – 4.9
Miscellaneous (n=49)	20% (n=10)	2.1	0.8 – 5.1
Culture negative (n=39)	26% (n=10)	2.8*	1.1 – 7.0
S. aureus (n=168)	52% (n=87)	8.5*	4.4 – 16.8

** $p<0.05$.*

streptococci (15%, 7/47) and in *S. aureus* IE (14%, 24/168) compared to episodes of other etiologies (1%, 6/469, p<0.001). Cerebral hemorrhage occurred in 5% of culture-negative IE (2/39) and 5% of *S. aureus* IE (9/168), but only the latter was significantly different from the incidence of cerebral hemorrhage seen in IE of other etiologies (1%, 5/477, p=0.003) (paper II).

If the analysis of CVC frequency was performed on the total cohort of patients including possible IE cases, the proportion of patients with culture-negative IE suffering any CVC (including hemorrhage) was lower, since definite IE diagnosis in culture negative IE relies on signs of embolism and other minor criteria.

4.5 Echocardiography

Incidence of vegetations

Vegetations were established in 75% of the 760 episodes in the total cohort that were possible to evaluate for the presence of vegetation. A symptomatic CVC was diagnosed in 25% of the episodes with vegetation on echocardiography as compared with in 12% of episodes without detected vegetations, p<0.001. Among episodes of NVE (paper III), a statistically significant dif-

ference in CVC rate between episodes with and without vegetations was detected as well (27%, vs. 14%, p=0.003). In paper II, where definite IE episodes were studied separately, the trend was similar but the difference was no longer statistically significant (26% vs. 18%, p=0.06). This discrepancy could be explained by the construction of the modified Duke criteria, since an embolic event is considered a minor criterion for IE. A suspected IE episode without an echocardiographic major criterion is frequently classified as definite, owing to the presence of embolism together with e.g. positive blood cultures, a predisposing condition and fever. This implies a higher incidence of CVC among IE episodes without an echocardiographically detected vegetation, when only definite episodes are studied. In paper I, a vegetation was observed in 70% (42/60) of the patients. A symptomatic CVC occurred in 45% of the patients with a vegetation, as compared with in 11% of patients without a vegetation, p=0.02.

Size of vegetation

IE episodes complicated with symptomatic CVC had significantly longer vegetations, median 10 mm (IQR 6-16) vs. 8 mm (IQR 5-12), p<0.001, when the total cohort was analyzed. This difference was statistically significant among definite IE episodes in paper II (median 10 mm [IQR 6-16] vs. 8 mm [IQR 5-12], p<0.001) and among NVE cases studied in paper III (median 11 mm [IQR 6-16] vs. 9 mm [IQR 5-13], p=0.006). Vegetation length differed in all types of CVC as compared with episodes without CVC except for TIA, where median vegetation lengths were similar in cases with and without the complication. Between IE cases with cerebral hemorrhage and cases without any CVC the difference in vegetation length did not reach statistical significance, which was probably an effect of the limited number of patients with this complication (data not shown).

In paper III the vegetation length in NVE episodes was found to be an independent predictive factor for CVC using a multivariable model. After adjustment for age, sex, *S.aureus* etiology, history of congestive heart failure or previous IE, and CRP level, vegetation length remained significantly associated with a higher incidence of CVC with an adjusted OR of 1.04 (95% CI 1.01-1.07, p=0.005).

In paper I there was also a significant association between vegetation length and incidence of both symptomatic and total cerebral embolism (including silent emboli). Median vegetation length in episodes with symptomatic CVC as compared with episodes without was 12 mm (IQR 8-16) vs. 8 mm (IQR 6-11), p=0.001. A similar correlation was seen for the incidence of total CVC (10 mm |IQR 8-15] vs. 5 mm [IQR 4-10), p<0.001). In multivariable logistic regression analysis the only variable correlated to an increased risk of both clinically symptomatic CVC (adjusted OR 1.17 [95% CI 1.05-1.30],

p=0.005) and total CVC (adjusted OR 1.23 [95% CI 1.08-1.39], p=0.001) was the length of vegetation. As mentioned above, *S aureus* etiology also conferred a higher risk for clinical cerebral embolism but not total cerebral embolism. In the multivariable analysis, the area under the ROC curve was calculated for the discriminating variables (weighted sum of vegetation length and *S. aureus* etiology, and only vegetation length) and found to be 0.811 and 0.782, respectively.

Additional aspects on echocardiographic findings

In 37 patients the echocardiographic examinations were inconclusive regarding the presence of vegetations, e.g. in cases with severe regurgitation and perforated heart valves, intracardiac abscesses or concomitant ruptured chordae tendinae. These episodes were regarded as "missing values" in the analysis of the relationship between the presence of vegetation and the incidence of CVC. Five of the episodes were classified as possible, 22 were definite according to surgical findings, six were definite attributable to the presence of an intracardic abscess, and the remaining four patients were classified as definite without the echocardiographic criterion fulfilled. In another 65 cases the echocardiographic examinations (all TEE) defined the presence but not the size of vegetation. All these cases were classified as definite, and 31 underwent cardiac surgery. The remaining 34 episodes were seen in patients with varying types of predisposing cardiac valve anomalies. Paravalvular involvement was found in 112 (14%) of the IE episodes, in 63% of these together with a vegetation. Paravalvular complications were significantly more frequent in PVE than NVE (p<0.001).

Multivariable modeling

After multivariable analysis (paper III) additional variables found to correlate to CVC incidence among NVE patients were history of congestive heart failure (aOR 0.22; 95% CI 0.1-0.52) and history of previous IE episode (aOR 0.1; 95% CI 0.01-0.79), both associated with a lower incidence of CVC.

4.6 Silent cerebral embolism

In paper I the incidence of silent CVC was found to be 30% (18/60) when MRI of the brain and CSF analysis were used to detect lesions (figure 7). The MRI lesions in patients with silent cerebral embolism were all small ischemic infarctions with a maximum size of 1 cm, and the findings were multiple in 40% of the patients. Out of the 27 patients with no IE related pathological MRI findings, 14 had other types of lesions, mainly minor old infarctions or white matter lesions.

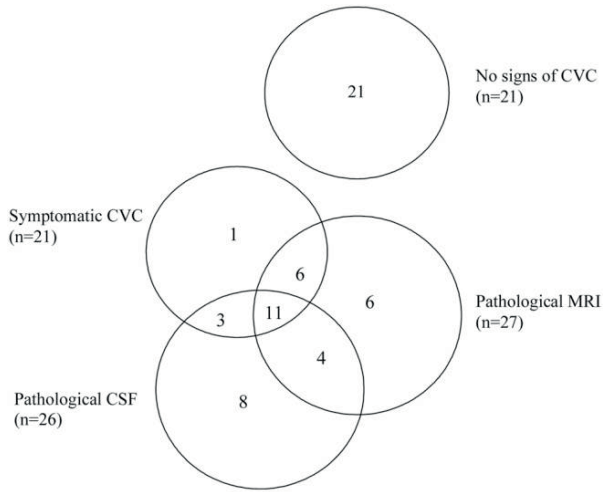

Figure 7. Incidence and distribution of cerebrovascular complications among 60 IE patients (paper I). Circles represent the patients with cerebrovascular complications detected by each method.

Only one of the patients in the study had a history of previous stroke, and no clinical or silent signs of CVC correlated to the current IE episode were detected in this patient. In one patient, previously operated on for falx meningeoma, minor residual meningeomal tissue and gliosis were found on MRI in combination with a new ischemic infarction in the cerebellum. In another patient a Chiari malformation not previously known was found, and this patient also had signs of silent CVC verified by both MRI and CSF findings. In yet another patient with clinically obvious cerebral embolism also verified with MRI and CSF findings, a previously diagnosed hypophyseal adenoma was seen. None of the patients in the study had a history of or signs attributable to other neurological diseases known to be associated with elevations in brain damage markers or specific MRI lesion (131, 133, 138).

Elevated levels of NFL (figure 8) and/or GFAP (figure 9) in CSF as markers of parenchymatous brain damage was seen in 24 patients, 12 of whom had no concomitant neurological signs. In all symptomatic patients both CSF-NFL and CSF-GFAP were elevated above the reference levels, and elevated levels of polymorphnuclear cells were found in seven patients as well. Among the 12 patients with silent cerebral embolism detected on the basis of CSF findings, eight were detected from elevations in brain damage markers only (CSF-NFL and CSF-GFAP elevated in five, only CSF-NFL in three). There were no statistically significant differences between CSF-NFL or CSF-GFAP levels in patients with symptomatic and silent CVC, respec-

tively, but for CSF-NFL the highest values were seen in symptomatic patients. For CSF-GFAP the highest value was detected in a patient with silent CVC and no ischemic lesion identified on MRI.

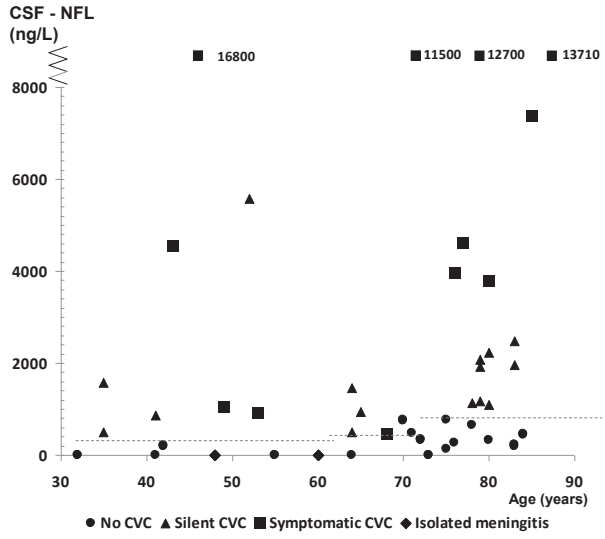

Figure 8. CSF-NFL levels in infective endocarditis patients according to presence and type of cerebrovascular complication (CVC).
Reference levels: < 59 years: < 250 ng/L, 60-69 years: < 380 ng/L, > 70 years: < 750 ng/L

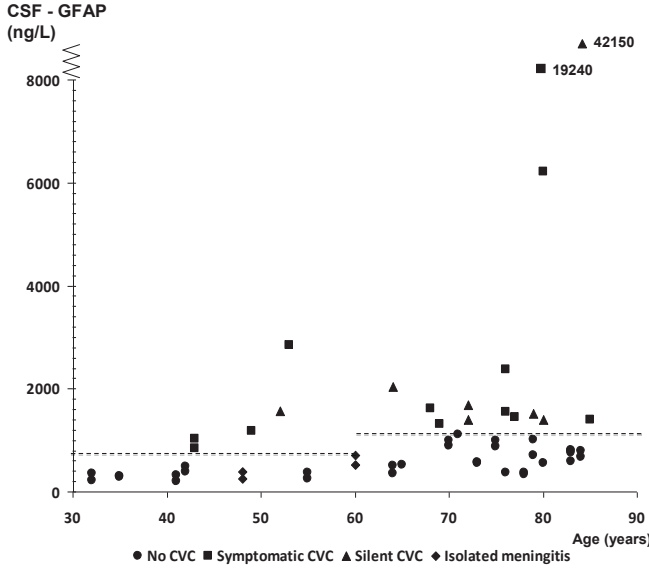

Figure 9. CSF-GFAP levels in infective endocarditis patients according to presence and type of cerebrovascular complication (CVC).
Reference levels: 21-59 years: < 750 ng/L, > 60 years: < 1250 ng/L

The kinetics of NFL and GFAP elevations in CSF after acute CNS damage differ considerably, with the highest CSF-GFAP elevations detected after 24-48 hours and normalization within less than three weeks. For CSF-NFL there is a gradual increase peaking after a few weeks and normalizing months after the acute insult. This is consistent with the isolated CSF-NFL increment found in three neurologically asymptomatic patients, where the assumption is that the expected concomitant GFAP elevation had already passed when the sample was taken. In the neurologically symptomatic patients the first LP was probably performed closer in time to the embolic event as compared with in the patients with silent CVC.

No LP was performed in 21 patients for reasons of concomitant anticoagulation (9), other contraindications (4), early surgery before LP was possible (3), refusal (3), and technical problems (2). CSF findings were normal in 13 patients.

4.7 Mortality

In-hospital mortality was 13% in the total cohort, 14% among definite cases (paper II) and 12% among NVE cases (paper III). Infective endocarditis patients with cerebral manifestation showed significantly higher in-hospital mortality than patients without such complications, 27% vs. 9%, $p<0.001$ (total cohort). Of all patients who died during hospitalization (104) for IE, 45% had a CVC prior to succumbing. The different in-hospital mortality between patients experiencing neurological complications were statistically significant when infarctions (30% vs. 9%, $p<0.001$) and cerebral hemorrhage (38% vs. 9%, $p=0.006$) were analyzed separately. For episodes complicated with meningitis the picture was different, and in patients with meningitis as the sole neurological manifestation, no significantly different in-hospital mortality was seen compared to patients without any CVC (11% vs. 9%, n.s.). For patients with isolated TIA there was a trend toward higher mortality (18% vs. 9%, n.s.). In paper I in-hospital mortality was only 2%.

In-hospital mortality was significantly correlated to age and increased for each 10-year interval from 50 to 70 years of age (<50 years: 5%, 51-60 years: 11%, 60-69 years: 14%, >70 years: 17%, $p=0.002$). Higher in-hospital mortality was seen for patients with CVC in all age groups, but for patients <50 years this trend was not significant (10% vs. 4%, n.s.). In-hospital mortality has been shown to correlate to age and CVC rate in other studies (39, 41, 42, 52, 150, 157).

Long-term outcome

Cerebral complications influenced long-term outcome. Among the 684 episodes of definite IE a survival analysis using Kaplan-Meier procedure showed a significantly lower survival rate among patients with CVC during the follow-up period (log rank test, p=0.004) (figure 10). Follow up varied from 114 days to 4473 days (12.2 years) with a median of 3.6 years. After 10 years the survival rate was 35% among patients who experienced a CVC during the initial IE episode and 50% in patients without CVC. The analysis after 10 years was based on 25 survivors of 589 alive at hospital discharge. The detrimental long-term impact of neurological complications has been shown in other studies (40, 72, 78) but the low 10-year survival rates in all patients also reflects the high median ages in IE patients in the present series.

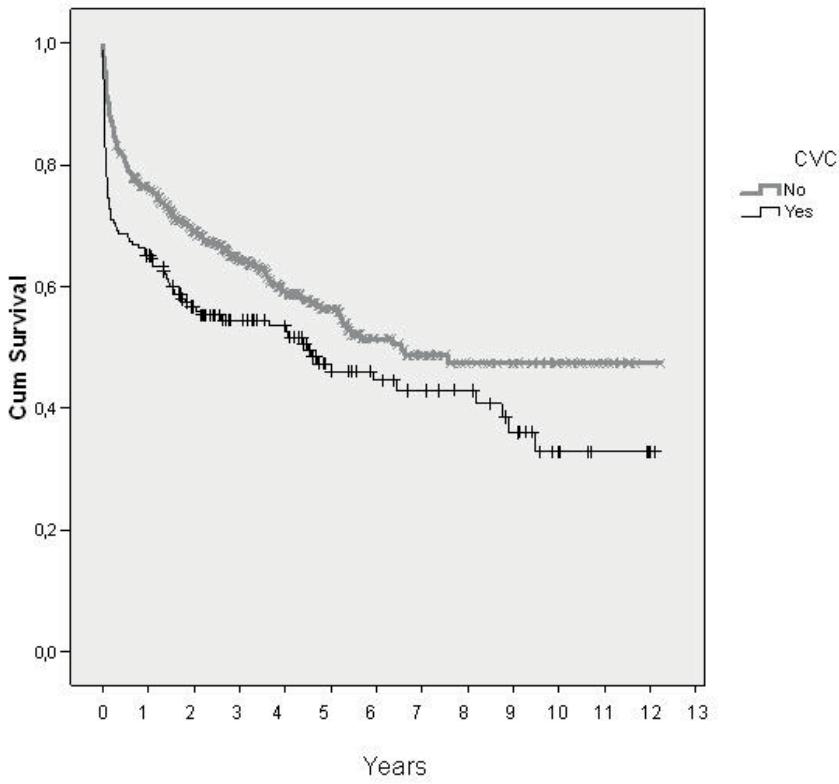

Figure 10. Kaplan-Meier analysis of long-term survival among IE patients with and without a cerebrovascular complication during the initial IE episode.
CVC: cerebrovascular complication, Cum survival: cumulative survival rate.

5 General considerations

Incidence of symptomatic cerebral manifestations

The symptomatic cerebral complication rate of 22% found in this large cohort of adult IE patients, with ischemic stroke as the dominant lesion, is in accord with recent studies focusing on cerebral complications (48, 49, 72). The higher incidence of symptomatic CVC found in paper I might reflect more sensitive clinical examinations in that study as well as a higher awareness of neurological symptoms in IE patients during the study period, but inclusion bias could not be ruled out. Unchanged frequencies of cerebral complications have been asserted since the preantibiotic era of IE, but figures are not comparable without further evaluation of the demographic characteristics of patients included in the different studies. The evolving epidemiology and varying definitions of IE and of cerebral complications, changing diagnostic procedures, different study designs and the influence of selection bias have to be considered in these comparisons.

In the preantibiotic era the proportion of IE episodes caused by streptococci, especially the viridans group streptococci but also beta-hemolytic streptococci and pneumococci, was much higher, and the natural history of the disease could be observed in all patients, since no therapeutic measures were possible. The rate of cerebral complications in IE episodes caused by viridans group streptococci was around 40-50% in studies prior to the mid-1940s (92, 146), which is comparable with the CVC rate found in *S. aureus* IE in our cohort, but much higher than the rates of CVC seen in viridans group streptococcal IE today. The enhanced possibilities for early diagnose of IE and for effective treatment have probably decreased the rate of CVC in streptococcal IE.

Regarding *S. aureus* IE, the development is less clear since the relative and probably also the absolute incidence of this disease has increased since the introduction of penicillin (34, 158) parallel to enhanced detection of IE and improved initial management of septic patients. Cerebral complications in *S. aureus* IE are high in modern studies (34, 55, 159) and were also described as high in early IE patient series (46). The improved diagnostic methods might have given a paradoxical increase of cerebral events in *S. aureus* IE,

since more patients with an inherently high risk of CVC survive the early septic phase and are therefore diagnosed with IE.

Cerebrovascular complications

In the present studies, all CNS complications were regarded as eminating from a cerebrovascular pathogenesis, based on the assumption that cerebral involvement in IE is mainly caused by septic micro- and macroembolism from valvular vegetations. Ths view has also been employed by others (94, 95). Cerebral emboli have been shown to cause a variety of pathogenetic processes and a variable pattern of often coexisting cerebral injuries (47, 89, 90, 92, 160). Our findings of multiple cerebral lesions are in accord with earlier observations. However, the incidence of stroke within the first month after a bacteremia, also without concomitant IE, has been shown to be higher than in the general population (161, 162). Complementary pathogenetic mechanisms, such as the presence of a hyperocoagulable state (163) or the presence of anti-phospholipid antibodies (164) have been suggested.

Silent cerebral embolism

Silent embolism both to the brain and to other parts of the vascular tree has been shown in previous studies (47, 56, 58, 66). However, the number of silent CVCs found in our study was higher than in previous studies. Our findings are in accordance with the reported findings in an ongoing French study also using MRI of the brain in IE patients to detect silent cerebral embolism and to assess the diagnostic and therapeutic impact of MRI findings. Interim analysis of the MRI findings in 130 IE patients in that study showed an even higher incidence of total CVC (symptomatic and silent events), reaching 82%, with 12% of the included IE patients experiencing concomitant neurological symptoms (pers. comm. Xavier Duval). The MRI protocol used in the French study differed from the protocol in our series, and they report multiple microbleeds as a typical finding in IE patients.

The impact of silent embolism on mortality was not analyzed in paper I, but the prognostic influence of silent cerebral embolism has been evaluated by Thuny et a. (72) in a French prospective observational study including 496 left-sided definite IE patients from 1990-2005. Cerebral CT scan was used systematically in all patients from 1993. In that study IE patients with silent CVC and TIA had a mortality risk not significantly different from IE patients without CVC, while patients with symptomatic stroke had significantly higher in-hospital and long-term mortality. Stroke remained a significant predictor of death after adjustment for other factors found to correlate to mortality.

In our total cohort of patients the in-hospital mortality in IE cases complicated with isolated meningitis was not significantly higher than in patients

with no cerebral complications, which supports the theory that the size and type of CNS damage is a main prognostic factor. In another prospective study published by Millaire et al. in 1997 (66), with systematic search for cerebral and peripheral emboli in IE, embolic events of all types were found in 51% of the patients but were not associated with significant attributable mortality. It has also been shown that IE patients with large symptomatic preoperative cerebral emboli have a worse prognosis after cardiac surgery than IE patients with small symptomatic lesions (52). The results in our studies verify previous findings showing that both symptomatic and silent cerebral complications in IE are common and that symptomatic events significantly influence in-hospital and long-term outcome while the prognostic impact of silent cerebral embolism is not clear. Potential improvements in the management of IE after detection of silent CVC have been suggested, e.g. modifications of antibiotic treatment (use of antibiotics with high CNS penetration), surgical management (episodes with large vegetations and silent CVC) or possible modifications in anticoagulant treatment. However, present evidence does not favor the need for routine search for asymptomatic cerebral complications in IE, but further investigations will guide future recommendations.

Antiplatelet therapy

Potential effects of antiplatelet agents, mainly ASA, in IE have been suggested since the late 1970s (114). Animal models of IE have outlined several protective effects of antiplatelet agents during IE, e.g. impaired vegetation growth, enhanced sterilization of vegetations, reduced adherence of *S. aureus* to sterile vegetations after preexposure to ASA, and down regulation of virulence factors (109, 110, 113, 165). A prospective randomized controlled trial of the effect of high-dose ASA (325 mg) initiated after IE diagnosis in 60 patients showed no reduction of systemic embolic events as compared with the frequency of embolic events in 55 IE patients given placebo (117). On the contrary, a retrospective analysis of embolic events in 600 IE patients found a significantly lower embolic frequency in patients with established use of antiplatelet agents during the development of IE (53). Three additional studies did not verify a significantly lower incidence of embolic events in patients on established antiplatelet agents (51, 118, 119). However, reduced three-month mortality in patients on previously established antiplatelet therapy was found in one of the studies, and a reduced need for cardiac surgery was found in another. These effects were postulated to be specific for *S. aureus* IE. A reduced incidence of catheter-associated *S. aureus* bacteremia has also been found in hemodialysis patients treated with 325 mg ASA, implicating a clinically useful anti-staphylococcal effect of ASA (166).

In the analysis of CVC incidence in paper II no correlation was found between prior established use of antiplatelet therapy and the frequency of CVC, and unadjusted 12-month mortality was significantly higher in patients on antiplatelet agents. However, when adjusted for other variables significantly associated with 12-month mortality, no significant relationship remained between 12-month mortality and antiplatelet therapy. Our findings thus do not support the hypothesis that antiplatelet therapy influences embolic tendency in IE patients, and no statistically significant conclusions could be drawn regarding the influence of antiplatelet therapy on mortality. However, important variables with previously shown major impact on mortality in IE, e.g. development of new or worsening congestive heart failure during IE, were not retained in our model. Additionally, the study was not designed to evaluate other possible benefits of antiplatelet therapy in IE. Despite the large number of patients in our study, the number of *S. aureus* IE episodes might have been too small to evaluate specific interactions between *S. aureus* and antiplatelet agents.

Hemorrhagic cerebral complications

Hemorrhagic complications were few in the present series, reaching 2% symptomatic bleeding events both in the total cohort of IE patients and in the patients systematically investigated using MRI and CSF analysis in paper I. No increase was detected among patients on antiplatelet therapy or oral anticoagulants. The number of detected ruptured cerebral mycotic (infectious) aneurysms was less than 0.5% in the total cohort of IE patients and only one patient with an unruptured aneurysm was found. Similar low rates of ruptured mycotic aneurysms have been found in other recent studies (49, 54, 72), but data supporting the occurrence of a considerably higher number of unruptured and clinically silent aneurysms that heal during antibiotic therapy have also been presented (47, 167, 168). The detected number of silent aneurysms will probably increase in the future following the use of further enhanced neuroradiological imaging, such as e.g. MR angiography. The low but prolonged risk of aneurysm rupture after antibiotic therapy has been completed, as seen in one of the patients in our study, is well recognized in previous studies (76, 151).

Oral anticoagulant therapy

The proportion of NVE patients on warfarin who suffered a CVC was significantly lower than the proportion of patients not on warfarin who suffered a CVC (6% vs. 26%, p=0.006). This was an unexpected finding since the only previous study separately addressing the issue of anticoagulation in NVE patients (96) found cerebrovascular accidents significantly more frequently among anticoagulated patients as compared with non-anticoagulated patients. The CVC proportion in that restrospective study remained significantly higher among anticoagulated NVE patients when cerebral hemor-

rhagic complications were analyzed separately, but not when ischemic cerebral complications (including CVC of unclear origin but not TIA) were analyzed. Among 269 NVE patients in that study, including patients from 1970-1987, cerebral hemorrhagic complications were seen in 5% and cerebral ischemic infarctions in 7% of the patients. The number of ischemic events was relatively low as compared with most other series, which implies very restricted diagnostic criteria for ischemic cerebrovascular accidents or possible selection bias.

Other previous studies addressing the relationship between anticoagulant therapy and cerebral embolic complications in IE patients have included very few anticoagulated patients (47) or have mainly focused on patients with PVE (97, 169-171). These studies have included patients over an extended period of time, and Leport et al. (169) found better management of anticoagulant treatment among patients in the later part of the study. It is not known whether improved general management of anticoagulant therapy in IE patients has since been achieved. One recently published study primarily addressing the effects of antiplatelet therapy in IE (118) detected a significantly higher incidence of hemorrhagic stroke among patients who were on warfarin prior to IE diagnosis, although no more detailed information was given. An increasing frequency of anticoagulant associated intracerebral hemorrhage was reported during the 1990s in patients with anticoagulant therapy for any indication (172).

The lower CVC rate among NVE patients on warfarin therapy in our study remained significant after adjustment for microbiological etiology and vegetation length, indicating that warfarin treatment may diminish the liability for embolization independently from vegetation length. Fibrin formation plays important roles during the development of IE, both in the attachment of bacteria to the surface of the vegetations and in the formation of a protective layer against host defense mechanisms within the vegetation. A disturbance in fibrin formation could therefore possibly influence both the development of IE and the frequency of embolism. Only a few experimental studies with diverging results were published in this area in the 1970s (7), while the question has not been addressed in later studies and merits further attention in future studies.

6 Conclusions

- Cerebrovascular complications were seen in 65% of patients with left-sided infective endocarditis when sensitive diagnostic methods were used, but almost half of these were clinically silent.

- Prior established antiplatelet therapy gave no protection against cerebral complications in infective endocarditis. After adjustment for covariables antiplatelet therapy had no significant impact on mortality.

- Ongoing oral anticoagulation with warfarin on admission for native valve endocarditis was associated with lower risk of cerebrovascular complications.

- *Staphylococcus aureus* etiology and vegetation length as detected using echocardiography were each correlated to a higher risk of cerebrovascular complications in infective endocarditis.

- Cerebral hemorrhagic complications were few. Antiplatelet therapy, warfarin therapy and prosthetic valve endocarditis conferred no higher frequency of these complications.

7 Svensk sammanfattning

Infektiös hjärtklaffsinfektion är en relativt ovanlig men livshotande sjukdom. I Sverige diagnosticeras cirka 550 fall årligen. Risk för hjärtklaffsinfektion är högre hos individer med skador på hjärtklaffarna eller inopererade hjärtklaffsproteser. Personer med kvarliggande intravenösa infarter, t ex hemodialyspatienter, har också förhöjd risk liksom individer med intravenöst missbruk.

Vid hjärtklaffsinfektion finns det bakterier i blodet, som fått fäste på en hjärtklaff och gett upphov till infekterade koagel där, s.k. vegetationer. Hjärtklaffen kan skadas och börja läcka, vilket kan medföra hjärtsvikt. Akut hjärtkirurgi behövs i c:a 30% av alla fall. En annan vanlig och allvarlig komplikation vid hjärtklaffsinfektion är s.k. embolisering, då små infekterade fragment från vegetationerna lossnar och förs med blodet till olika organ med åtföljande skador. Detta sker i 20-60% av episoderna och hjärnan drabbas mest frekvent, vilket medför olika typer av neurologiska symtom och skador. Kliniskt tysta (asymtomatiska) embolier förekommer också. Skadorna i hjärnan är av varierande typ. Vanligast är hjärninfarkt orsakad av förhindrad blodförsörjning till en del av hjärnan, men infektion i hjärnan eller hjärnhinnorna och hjärnblödning förekommer också. Olika typer av skador kan uppstå parallellt.

Syftet med avhandlingens studier var att undersöka frekvensen symtomgivande och tysta embolier till hjärnan samt att kartlägga faktorer associerade med ökad och minskad emboliseringsrisk. I delstudie I undersöktes 60 patienter med magnetresonanstomografi (MR) och provtagning av ryggmärgsvätska med analys av markörer för hjärnskada. Andelen patienter som drabbades av symtomgivande neurologiska komplikationer var 35% och därutöver påvisades tyst embolisering till hjärnan hos ytterligare 30%.

I delstudie II undersöktes om mediciner som minskar blodets levringsförmåga via hämning av blodplättarnas funktion (vanligen acetylsalicylsyra, ASA) minskar risken för symtomgivande embolisering till hjärnan. Andelen patienter som drabbades av neurologiska komplikationer var likartad hos patienter med och utan blodplättshämmande medicinering.

I delstudie III påvisades en minskad andel symtomgivande embolier till hjärnan hos patienter som stod på blodförtunnande medicinering i form av warfarin, ett potent antikoagulerande läkemedel som verkar via minskad produktion av ett flertal koagulationsfaktorer. Hjärtklaffsinfektion orsakad av *Staphylococcus aureus* samt större uppmätt vegetationslängd medförde större risk för neurologiska komplikationer.

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